Determination of Clonazepam in Tablets by dc Polarography

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Abstract □ A simple and convenient method for the routine determination of clonazepam in tablets by dc polarography is described. Clonazepam is extracted from the sample by ethanol and diluted with pH 4.15 acetate buffer in a volumetric flask. The filtered solution is then polarographed at the dropping mercury electrode *versus* the saturated calomel electrode. The polarographic wave is well developed; the determination is quantitative, precise, and accurate.

Keyphrases □ Clonazepam—dc polarographic analysis in tablets □ dc Polarography—analysis, clonazepam in tablets □ Anticonvulsants—clonazepam, dc polarographic analysis in tablets

Several methods, *e.g.*, electron-capture GLC (1–7) and radioimmunoassay (8), have been reported for the determination of clonazepam. These methods, although highly sensitive, are time consuming and require meticulous handling and skilled technical operations.

Polarographic reduction of the nitro group of clonazepam in whole blood has been reported (9). Differential pulse polarographic analysis of major urinary metabolites of clonazepam has been accomplished by reduction of the azomethine group (3).

This study was undertaken to develop a simple, rapid, and reliable dc polarographic analysis for clonazepam[1], 5-(2-chlorophenyl)-1,3-dihydro-7-nitro-2*H*-1,4-benzodiazepin-2-one, in tablets. Properly applied, the polarographic method is stoichiometrically valid and reproducible.

EXPERIMENTAL

Apparatus—A recording polarograph[2] equipped with a polarograph stand, which includes the dropping mercury electrode, a saturated calomel electrode, and a nitrogen bubbler placed in a standard cell, was used.

Reagents and Chemicals—A pH 4.15 acetate buffer was prepared by mixing 41.0 ml of 0.2 *M* acetic acid with 9.0 ml of 0.2 *M* sodium acetate.

For the stock standard solution, 60 mg of clonazepam standard was dissolved in 190 ml of 95% ethanol in a 200-ml volumetric flask and diluted to volume with the same solvent.

Sample Preparation—Twenty tablets were accurately weighed, and the average weight per tablet was determined. These tablets were finely ground, and the equivalent of tablet powder containing 2 mg of clonazepam was accurately weighed. The powder was transferred into a 100-ml volumetric flask, 10 ml of 95% ethanol was added, and the flask was shaken for about 10 min. Then 50 ml of acetate buffer was added; the flask was shaken for another 10 min and diluted to volume with the buffer. The solution was filtered through filter paper[3], discarding the first 20 ml of filtrate.

Polarography—The filtered solution (25 ml) was transferred into the polarographic cell, and oxygen-free nitrogen was bubbled through it for 10–15 min. The polarogram was recorded from 0.00 to −0.5 v (applied voltage on the dropping mercury electrode *versus* the saturated calomel electrode). The other conditions were: mercury height, 45 cm; drop time, 2.8 sec; and sensitivity, 1×10^{-8} amp/mm of the scale of the recorder. A suitable damping was applied. The height of diffusion current (i_D) was measured. The standard clonazepam solutions, 1, 1.5, 2, 2.5, and 3 mg/100 ml of pH 4.15 acetate buffer containing 10% of 95% ethanol, were polarographed under the same conditions, and the wave heights were measured. A calibration curve, diffusion current *versus* concentration, was then established.

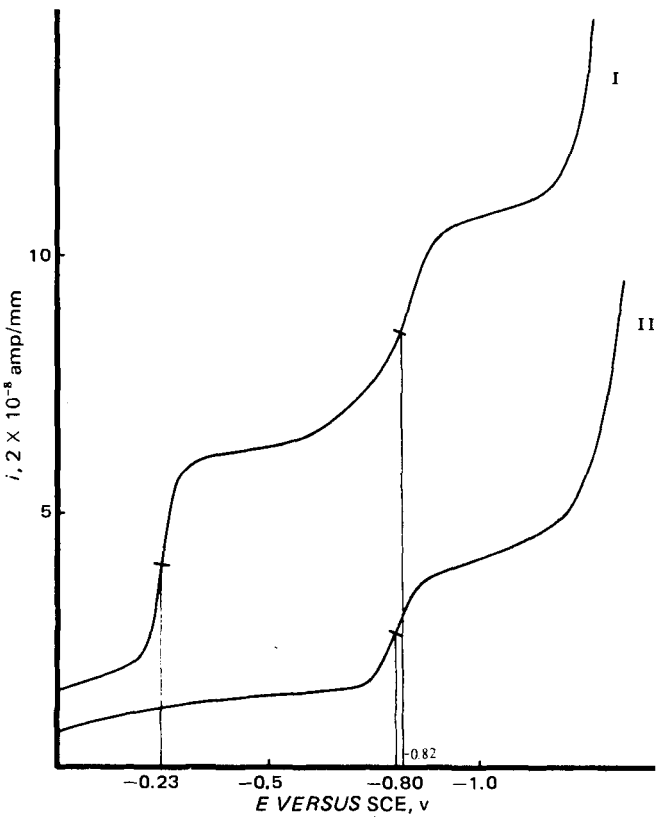

Figure 1—*Polarograms of clonazepam (I) and diazepam (II) in acetate buffer–ethanol.*

Calculations—By referring the diffusion current, i_D, measured for the sample to the calibration curve, the amount of clonazepam, C_x, in the weight of sample taken, W_s, can be found using:

$$\text{mg of clonazepam/tablet} = \frac{C_x \times W_t}{W_s} \qquad \text{(Eq. 1)}$$

where W_t is the average tablet weight.

RESULTS AND DISCUSSION

When clonazepam was polarographed in pH 4.15 acetate buffer, two reduction waves were observed. The half-wave potentials were −0.23 and −0.82 v (*versus* the saturated calomel electrode) (Fig. 1).

Table I—Diffusion Current at Different Concentrations of Clonazepam Standard

Clonazepam Concentration, mg/ml	i_D, 1×10^{-8} amp
0.030	118.5
0.025	97.0
0.020	76.5
0.015	56.5
0.010	37.0

[1] Rivotril, Hoffmann-La Roche, Vaudreuil, Quebec, Canada.
[2] Polarocord, type E 261 R, Methrohm AG, Herisau, Switzerland.
[3] S & S No. 595.

Table II—Analysis and Recovery of Clonazepam in Tablets

Assay before Addition of Standard				Assay after Addition of Standard			
Sample	Theoretical Concentration, mg/100 ml	Assay Value, mg/100 ml	% Theory	Sample	Theoretical Concentration, mg/100 ml	Amount Added, mg	Amount Found, mg
			2-mg Tablets				
A	2	1.91	95.5	F	1	1	1.96
B	2	1.90	95.2	G	1	1	1.96
C	2	1.92	96.0	H	1	1	1.96
D	2	1.93	96.5	I	1	2	2.93
E	2	1.89	94.5	J	1	2	2.93
Average		1.91		K	1	2	2.90
Range		1.89–1.93					
SD		1.58×10^{-2}					
			0.5-mg Tablets				
L	2.0	2.0	100.0	Q	1	1	2.0
M	2.0	2.01	100.6	R	1	1	1.99
N	2.0	2.01	100.7	S	1	1	1.98
O	2.0	2.01	100.7	T	1	2	2.97
P	2.0	2.01	100.7	U	1	2	2.96
Average		2.008		V	1	2	2.98
Range		2.0–2.01					
SD		1.08×10^{-2}					

Table III—Single-Tablet Assays of Clonazepam

2-mg Tablets				0.5-mg Tablets			
Sample	Theoretical Concentration, mg/100 ml	Assay Value, mg/100 ml	% Theory	Sample	Theoretical Concentration, mg/25 ml	Assay Value, mg/25 ml	% Theory
1	2	1.920	96.0	1	0.5	0.494	98.7
2	2	1.934	96.7	2	0.5	0.494	98.7
3	2	1.934	96.7	3	0.5	0.496	99.3
4	2	1.908	95.4	4	0.5	0.502	100.3
5	2	1.908	95.4	5	0.5	0.503	100.6
6	2	1.894	94.7	6	0.5	0.500	100.0
7	2	1.934	96.7	7	0.5	0.496	99.3
8	2	1.928	96.4	8	0.5	0.496	99.3
9	2	1.936	96.8	9	0.5	0.494	98.7
10	2	1.908	95.4	10	0.5	0.494	98.7
Average		1.920		Average		0.497	
Range		1.894–1.936		Range		0.494–0.503	
SD		1.50×10^{-2}		*SD*		3.48×10^{-3}	

The basic difference between clonazepam and diazepam is the presence of a nitro group on C-7 of the clonazepam structure while diazepam bears a chlorine atom at the same position. Both structures have a carbonyl group. When polarographed under the same conditions, diazepam gave only a single reduction wave with a half-wave potential of −0.80 v (Fig. 1). It may be assumed that both compounds undergo the same polarographic reduction of their respective carbonyl group. The first wave for clonazepam, absent with diazepam, may be attributed to the nitro group. This more positive wave (Fig. 2) was used for the quantitative determination of clonazepam. A plot of the diffusion current *versus* the concentration of clonazepam was linear in the range of 1–3 mg/100 ml (Table I). Maxima suppressors were not needed since no maximum was observed. Clonazepam is stable at pH 4.15. All operations were carried out at room temperature.

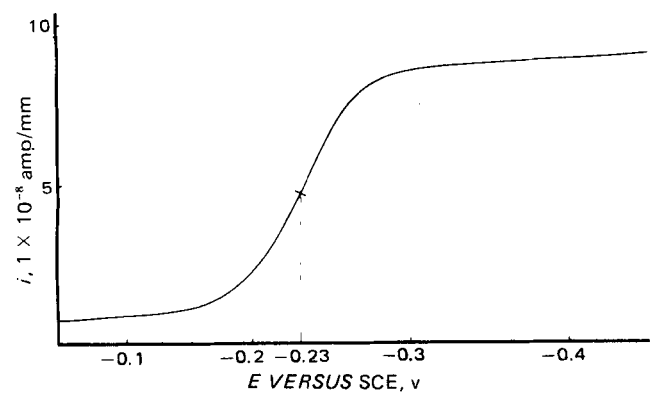

Figure 2—*Clonazepam reduction wave used for its assay.*

Each of the two dosage forms currently available was analyzed in two ways: analysis of clonazepam in the original unaltered dosage form and analysis of the sample after addition of a specific amount of clonazepam standard. This procedure was followed to ensure that the extraction was adequate and that all clonazepam had been dissolved and to test the accuracy of the polarographic method. The results are shown in Table II.

The wave obtained for clonazepam under the described conditions was well developed. As shown in Table II, clonazepam was quantitatively extracted and determined with excellent accuracy and precision without interference from any other component in the dosage form.

The method was also applied to a single-tablet assay (Table III).

This polarographic method is simple, rapid, and quite suitable for routine assay as well as for single-tablet assays.

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Solubility of Calcium Oxalate in 1-Alkanols and Ethanol–Water Mixtures

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Abstract □ Solubility profiles for calcium oxalate were determined in pure 1-alkanols and ethanol–water mixtures at 20°. The magnitude of solubility in the aliphatic alcohols was highest in methanol and decreased in a nonlinear fashion as the dielectric constant decreased, going to *n*-decanol. In ethanol–water mixtures, the solubility increased nonlinearly with water content or increasing dielectric constants. The work factor for these systems was also calculated and, as expected, a "mirror image" to the solubility profiles was evidenced. In the mixed solvent system, a dramatic change in the magnitude of solubility occurred after a dielectric constant value of about 60, indicating ionic association or ion-pairs below this value. The Born relationship of solubility and ionic size was tested in the ethanol–water system, and the ionic size of the divalent species of about 2.27 Å agreed with the theoretical ionic size of about 2.4 Å. These results indicate that the water number of calcium is 8 or that a monolayer of dipoles surrounds this divalent cationic species.

Keyphrases □ Calcium oxalate—solubility in various 1-alkanols and ethanol–water mixtures □ Solubility—calcium oxalate in various 1-alkanols and ethanol–water mixtures

Calcium oxalate, a component of certain types of "body" stones, is a typical example of a metallo-organic substance whose solubility characteristics should be a major area of investigation. This study is an initial effort into determining these solubility profiles in a series of pure 1-alkanols and ethanol–water mixtures. Since calcium oxalate is highly insoluble, its solubility as a function of polarity would be determined by covering the range of polarity by various solvents and solvent mixtures. Additionally, the spectrum of polarity would be manifested by the dielectric constants of the solvent systems. Thus, the charge-separating ability of various solvents would be indicative of their effect on the ionization and subsequent dissociation of the solute measured by the solubility product of calcium oxalate.

BACKGROUND

Calcium oxalate is considered to be insoluble in both polar and nonpolar solvents (1). It may occur as a mono-, di-, or trihydrate, with the monohydrate being the most stable form as well as the least soluble (2, 3). The monohydrate has a solubility product of 2.57×10^{-9} mole/liter at 25° (4).

In 1929, Hammarsten (5) studied the solubility of calcium oxalate, applying the Debye–Huckel theory of ion activity in the presence of urinary electrolytes. A "salting-in" increase in solubility was found by the use of sodium chloride, monobasic sodium phosphate, potassium chloride, and magnesium chloride. Also, pH had no effect when varied over the usual physiological range.

Shehyn and Pall (6) studied the solubility of calcium oxalate as a function of the ionic strength of various salts. Other studies relating to solubility in salt solutions also were performed (7–9). Finlayson *et al.* (10) presented information on the complex set of equilibria of calcium oxalate in aqueous systems. An ionizable salt, sodium salicylate, was studied previously in mixed solvent systems of various types (11).

EXPERIMENTAL

Chemicals—Calcium oxalate[1], calcium chloride[2], methanol[3], ethanol[4], 1-propanol[5], 1-butanol[6], 1-pentanol[7], 1-hexanol[8], 1-octanol[9], 1-decanol[10], and distilled deionized water were used. The purity and anhydrous nature of the solvents were tested as previously described (11).

Equipment—A refractometer[11], a demineralizer[12], a centrifuge[13], and an oscillometer[14] were used.

The refractometer was used to check the purity of the solvents, and the oscillometer was used to determine dielectric constants.

Procedure—A rotating apparatus holding screw-capped glass vials was filled with the appropriate solvent or solvent mixture and rotated at 40 rpm in the presence of excess calcium oxalate. The temperature was maintained at 20° by a temperature control unit[15]. Samples were withdrawn with pipets through a glass wool wrap after equilibration for 24 hr. The samples were centrifuged at 1000 rpm for 10 min because of the fine nature of particles, and a clear supernate at 20° was drawn for analysis on an atomic absorption spectrophotometer[16].

The samples were analyzed from previously determined calibration curves with known concentrations of calcium as calcium chloride in the appropriate solvents or solvent mixtures. These measurements were made with a calcium vapor lamp at the 4227-Å absorption line for gaseous calcium atoms at a source current of 12 μamp using an acetylene–air mixture.

For the pure solvents and up to about 40% water in the mixed solvent system, 12 samples were analyzed. At higher water concentrations to pure water, eight samples were tested for calcium concentration. These results

[1] Lot 762198, certified powder, J. T. Baker Chemical Co.
[2] Lot XGZ, anhydrous, Mallinckrodt Chemical Works.
[3] Lot VMN, anhydrous, spectrophotometric grade, Mallinckrodt Chemical Works.
[4] Absolute, U.S. Industrial Chemical Co.
[5] Lot 35592, "Baker Analyzed," J. T. Baker Chemical Co.
[6] Lot TDY, analytical reagent, Mallinckrodt Chemical Works.
[7] Lot 776291, certified, U.S. Industrial Chemical Co.
[8] Lot H1330, Aldrich Chemical Co.
[9] Lot 22, Mallinckrodt Chemical Works.
[10] Lot 17, Matheson, Coleman and Bell.
[11] Abbe-3, Bausch & Lomb Optical Co.
[12] Bantam model BD-1, Barnstead Still and Sterilizer Co.
[13] Damon/IEC Division, Damon Corp.
[14] Chemical model V, E. H. Sargent and Co.
[15] Temptrol 150, Precision Scientific Co.
[16] Model 3D3, Perkin-Elmer Corp.